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Genetic Line Differences in Cytokine mRNA Expression of Peripheral Blood Leukocytes Exposed to Salmonella enteritidis In-Vitro

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Summary and Implications

Three genetically diverse chicken lines were used to evaluate genetic line differences and pathogen affect on early cytokine gene expression to *Salmonella enteritidis*. Cytokines are communication molecules in the immune system. Early cytokine involvement is critical to a host's ability to mount an effective immune response to *S. enteritidis*. Knowledge of genetic differences in cytokine expression will facilitate a greater understanding of host and pathogen dynamics, and may enable selection for enhanced disease resistance to this pathogen.

Introduction

Food safety concerns and movement away from feed additives has prompted researchers to examine mechanisms of disease resistance in food production animals to address these issues. Egg and carcass contamination in poultry is of serious concern. As *S. enteritidis* is often the source of such contamination, investigation of the host's immune response to this pathogen is warranted. A detailed picture of the bird's immune response to *S. enteritidis*, including cytokine expression, will provide assistance in promoting healthier animals and safer poultry products.

Materials and Methods

Two highly inbred lines of chickens (Leghorn and Fayoumi) and an outbred broiler line were evaluated for early cytokine expression. Peripheral blood leukocyte (PBL) samples were obtained from three adult male birds of each genetic line. Following culture for 2 or 4 hours, and with or without *S. enteritidis*, total RNA (genetic material) was isolated from these samples. mRNA expression levels of cytokines IL-2, IL-6, IL-8, IL-18, and a 28s reporter gene were evaluated by quantitative PCR utilizing SYBR Green dye technology. Plasmids containing each of the interleukin genes were used to generate gene-specific standard curves, later used for quantification purposes. Data were normalized to account for primer pair efficiency and starting template amount. Gene expression levels were represented both as cycle threshold (Ct) values and fold change.

Results and Discussion

Using Ct values, the effect of treatment (with or without *S. enteritidis*) was significant on mRNA expression of IL-6, IL-8, and IL-18. For each of these three cytokines, exposure to *S. enteritidis* resulted in a decrease in mRNA expression. In addition, a significant line by time interaction on IL-6 and IL-18 gene expression was observed (for Ct value). For both cytokines, the broiler and Leghorn lines showed an increase in expression from 2 to 4 hours. However, the Fayoumi line consistently showed decreased expression levels over these time points.

Gene expression differences in *S. enteritidis* and non- *S. enteritidis* were also measured and presented as fold changes (bacteria-exposed over non-exposed). Using fold change, the effects of line and time were significant for IL-6. The Fayoumi line showed a smaller IL-6 expression fold change for the comparison of *S. enteritidis* and non- *S. enteritidis* exposure than the broiler and Leghorn lines. IL-6 mRNA expression was reduced at the 4 hour time point compared to 2 hours when all lines were combined.

Future studies will include examination of additional cytokines and specific organs of immunological importance in these three genetic lines and advanced intercross lines.

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Table 1. Effects (*P* values) of line, time, and treatment on cytokine expression to in-vitro *Salmonella enteritidis* exposure, as expressed in Ct values.

Variable	IL-2	IL-6	IL-8	IL-18
Line	0.985	0.184	0.909	0.082
Time	0.862	0.259	0.423	0.351
Treatment	0.885	< 0.0001	< 0.0001	0.021
Line * Time	0.085	0.027	NS	0.030

Table 2. Effects of line and time on cytokine expression to in-vitro *Salmonella enteritidis* exposure, as expressed by fold change.

Variable	IL-2	IL-6	IL-8	IL-18
Line	0.267	0.045	0.396	0.340
Time	0.245	0.025	0.353	0.242
Line * Time	NS	NS	NS	NS